human malarias is being studied in a number of ecologically diverse situations in Malaya, to determine whether malaria is, under any circumstances, a zoonotic disease in nature.

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17 April 1961

Suppression of Shoot Formation in Cultured Tobacco Cells by Gibberellic Acid

Abstract. When tobacco pith was cultured on media containing gibberellic acid, shoot formation was observed. The formation of stem structures was strikingly suppressed by concentrations of 0.5 mg/lit. and above.

Application of gibberellins has generally resulted in promotion, rather than suppression, of plant growth processes. One of the few instances of suppression reported is in the rooting of cuttings. Gibberellin treatments not only inhibited rooting of cuttings, but also counteracted the stimulation caused by auxin (*1*). In tests performed with cultured tobacco cells, I found that gibberellic acid indeed prevented formation of roots under otherwise favorable conditions.

The present report considers the effect of gibberellin on the formation of stem structures in cultured cells. A preliminary study showed complete suppression of shoot formation when 5 mg of gibberellic acid per liter was included in the culture medium. Representative cultures can be seen in Fig. 1. The present experiment extends this observation to other concentrations of the growth regulator.

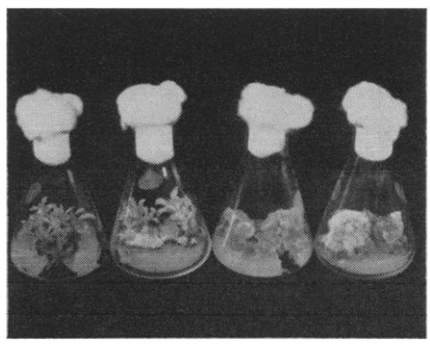

Fig. 1. Formation of shoots in cultured tobacco cells as influenced by gibberellic acid. Pair on left, no gibberellin; pair on right, 5 mg of gibberellic acid per liter.

Tobacco (*Nicotiana tabacum* L. var. Wisconsin 38) pith, which had been in continuous culture for the last several years at the University of Wisconsin, was employed (*2*). The basal medium of Miller *et al.* (*3*), supplemented with three times the level of inorganic phosphate, 0.5 mg of 3-indoleacetic acid, 2 mg of kinetin (*2*), and 150 mg of L-tyrosine per liter, was utilized. The reports of others (*4, 5*) and my experience showed that these levels of the supplements were optimum for shoot formation in cultured tobacco cells. Gibberellic acid (*6*) was included (0, 0.5, 1.0, 5.0 and 10.0 mg/lit.). Solutions of this compound were sterilized by Millipore filtration to avoid any undesirable consequence of heating.

For each level of gibberellin, ten 25- by 150-mm culture tubes, each containing 25 ml of medium, were employed. One piece of callus, roughly 2 mm^3 and weighing about 40 mg, was cultured in each tube. The cultures were maintained at 21° to 27°C under continuous weak light from overhead fluorescent fixtures.

The numbers of cultures with shoot and shoots per culture recorded after 7 wk are shown in Table 1. Controls on basal medium showed profuse shoot development, whereas cultures supplied with gibberellic acid in any concentration exhibited marked suppression. Levels of 5 and 10 mg/lit. of the compound were completely antagonistic. This suppression of shoot formation is most probably not due to toxicity, since no reduction in callus growth was observed in any of the treatments. Furthermore, the concentrations presently employed ranged below that found by Nickell and Tulecke (*7*) to be promotive in growth tests with cell cultures of a large number of species.

These findings, together with earlier reports of inhibition of rooting, show that gibberellin is indeed physiologically distinct from either auxin or kinin. Auxin tends to promote rooting, and kinin enhances shoot formation (*5*). If the amounts of gibberellin presently incorporated into the culture medium can be assumed to have resulted in levels which do not exceed the normal physiological range in the cells, then the data reveal that, whereas gibberellin is known to stimulate organ enlargement, the stem in particular, it antagonizes the initiation of the structure (*8*).

Table 1. Shoot formation in cultured tobacco pith as influenced by gibberellic acid.

Gibberellic acid in medium (mg/lit.)	Cultures with shoot (No.)	Shoots per culture (No.)
0	10	29 ± 4
0.5	3	0.6 ± .4
1.0	4	1.3 ± .6
5.0	0	0
10.0	0	0

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2. Both tobacco pith and kinetin were obtained through the courtesy of Folke Skoog, University of Wisconsin.
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28 February 1961

Acid-Catalyzed Oxidation of Reduced Pyridine Nucleotides

Abstract. Reduced pyridine nucleotides are oxidatively catalyzed in weakly acidic solutions. The rate is proportional to the acidity, and at constant acidic *p*H, the reaction follows first-order kinetics. The rate of oxidation of reduced triphosphopyridine nucleotide is approximately 3 times that of reduced diphosphopyridine nucleotide. The reaction offers a very plausible explanation for the metabolic efficiency of the malignant tumor cell. It may also play a key role in wound healing and muscle contraction.

When reduced pyridine nucleotides (DPNH and TPNH) (*1*) are dissolved in weakly acidic buffers, they are found to undergo catalytic oxidation which may be followed spectrophotometrically by the decrease in optical density at 340 mμ. If the concentration is expressed in terms of optical density, the reaction follows first-order

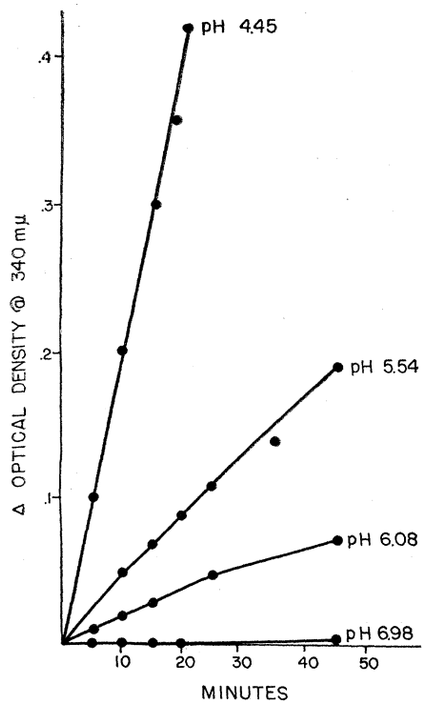

Fig. 1. Acid-catalyzed oxidation of DPNH.

kinetics at a given acidic *p*H. The *p*H dependency of the reaction rate is indicated in Fig. 1 for DPNH in phosphate buffers. The decrease in optical density is recorded as positive integers.

When the oxidative rates are compared for equivalent concentrations of DPNH and TPNH at constant acidic *p*H, the rate for TPNH oxidation is approximately 3 times that for DPNH oxidation. This is shown in Fig. 2.

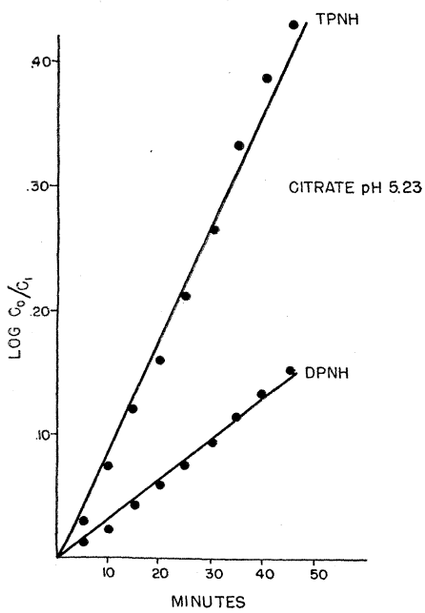

Fig. 2. Acid-catalyzed oxidation of TPNH and DPNH.

28 JULY 1961

Preliminary experiments suggest that the reaction does not involve heavy metal catalysis, for no inhibition is observed with 1×10^{-2} m*M* NaCN; nor is there any curtailment of activity noted in a metal-free buffer prepared from "tris" (tris-hydroxymethylaminomethane) and HCl. However, this aspect is being investigated more fully, since oxidative catalysis without heavy metal participation is indeed a singular event. Evidence thus far points to the pyridine moiety as being the sole locus of oxidation. The purine component remains unaltered, as evidenced by measurements at 260 mμ.

The biochemical importance of the reaction becomes apparent when it is recalled that TPNH exists in considerable excess over triphosphopyridine nucleotide in all tissues (*2*). The interpretation generally given is that the oxidation of TPNH is a rate-determining step in the various biosynthetic reactions in which it is linked (*3*). This is probably true for normal cells, which function in the *p*H range of 7.0 to 7.4. However, for malignant tumor cells, which metabolize in decidedly acidic *p*H (*4*), primarily because of accumulated lactic acid (*5*), the oxidation of TPNH would not be rate-limiting. This reaction therefore represents a means by which a tumor cell exercises metabolic superiority over the normal cell. It also explains the so-called "nitrogen trap" mechanism usually associated with tumors, for the accelerated TPNH oxidation would result in the sparking of biosynthetic reactions.

The reaction may also play a key role in wound healing and muscle contraction, processes in which there is a local sharp drop in *p*H (*6*). In wound healing, the injured tissue, aided by the nonenzymic oxidative catalysis, would emphasize the synthetic reactions of repair until the *p*H returns to neutrality. Lactic acid formed in muscle contraction would stimulate the TPNH-linked synthesis of glycogen. The difference between the malignant tumor and these two processes is that there is no return to neutrality in the case of the tumor (*7*).

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7. I acknowledge with gratitude the assistance of Marian Berman in preparing the figures.

5 April 1961

L-Tyrosine Oxidase System in Tuber of Nutsedge

Abstract. In the nutsedge, *Cyperus rotundus* L., the oxidation of L-tyrosine follows steps similar to those reported for mammalian liver and *Blattella conjuncta*. The system is activated by α-ketoglutarate, L-ascorbic acid, glutathione, and pyridoxal phosphate. Folic acid markedly inhibits the reaction.

Previous studies (*1*) have shown that the nutsedge tuber has an active tyrosinase. Ascorbic acid activated 3,4-dihydroxyphenylalanine (DOPA) oxidase, but ascorbate inhibited the complete oxidation of this compound to hallochrome. The dormant tuber contains large quantities of ascorbic acid (115 mg/100 g of fresh weight), and it is evident that this vitamin undoubtedly plays a vital role in the metabolism of the tuber. The function of ascorbic acid in the L-tyrosine oxidase system has been shown for mammalian liver (*2, 3*) and *Blattella conjuncta* (*4*).

The purpose of the present study was to follow the oxidation of L-tyrosine by nutsedge tuber tissue as influenced by α-ketoglutarate and certain cofactors.

Dormant nutsedge tubers were taken from greenhouse pots. The tubers were homogenized at 2°C in a chamber of the Omni mixer which contained 100 ml of 0.2*M* potassium phosphate buffer at *p*H 6.8. The suspension was strained through one layer of cheesecloth, and the filtrate was centrifuged at 1300*g* for 10 min at 2°C.

Activity was determined by conventional Warburg methods at 25°C. The vessels contained the cofactors and substrates in a total volume of 2.4 ml of 0.2*M* potassium phosphate buffer. A 0.6-ml portion of the homogenate was placed in the side arm. The reaction was initiated by tipping the side arm. The final *p*H for the treatments was